

Correlation between serum zinc levels and successful immunotherapy in recurrent spontaneous abortion patients

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Received: 06.10.2012
Review completed: 07.02.2013
Accepted: 09.06.2013

Access this article online

Quick Response Code:



Website:

www.jhrsonline.org

DOI:

10.4103/0974-1208.117170

ABSTRACT

BACKGROUND: Immunotherapy with paternal lymphocytes plays an important role in preventing recurrent spontaneous abortion (RSA) and is an effective treatment for it. This kind of treatment is performed as an immunotherapy method in several centers in the world. It attributes to the production of anti-paternal cytotoxic antibodies (APCAs) in women with RSA. Production of APCA after lymphocyte immunotherapy (LIT) in RSA patients gives them a better chance for successful pregnancy. Regarding the important effect of trace elements on the function of the immune system, we tried to investigate the correlation between serum zinc level and the success of LIT in RSA. **MATERIALS AND METHODS:** Serum zinc concentration was determined in two groups of RSA patients using atomic absorption spectrophotometer systems. Group (a) that responded to the paternal lymphocytes and their cross-match test was positive, and group (b) that had no response to the paternal lymphocytes immunizations and their cross-match test was negative. **RESULTS:** Serum zinc levels in group (a) patients were $74.98 \pm 11.88 \mu\text{g/dl}$, which was significantly higher than those in group (b) with the zinc concentration of $64.22 \pm 9.22 \mu\text{g/dl}$. **CONCLUSIONS:** Zinc deficiency may be one of the substantial causes of negative results for LIT in RSA patients. Therefore, compensation of zinc defect before LIT can be a promising approach to improve the immune response in patients.

KEY WORDS: Immunotherapy, recurrent spontaneous abortion, serum zinc levels

INTRODUCTION

Recurrent spontaneous abortion (RSA) is considered as three or more clinically detectable failure of pregnancy that occurs before 20 or even 28 weeks or abortion of fetus weighting $<500 \text{ g}$.^[1-4]

RSA is the most common complication of pregnancy with the involvement of 1 in 300 pregnant women.^[3] Several factors assume to have a correlation with RSA, including genetic factors,^[5,6] anatomical problems,^[7,8] endocrine hormones,^[9] infections,^[10] placental abnormalities, alcohol and tobacco, and exposure to environmental factors such as lead, ethylene oxide, ionizing radiation,^[11] and immunological factors.^[12-15]

Immunological relationship between mother and fetus is due to interaction of embryonic antigens and mother's immune system

that recognizes and reacts with foreign antigens. There is clear evidence that shows recognition of fetus antigens is essential for maintaining pregnancy and that inadequate recognition may lead to abortion in RSA women.^[3,16,17]

Many studies confirm the association between recurrent abortions and parent similarity of human leukocyte antigens (HLA). This similarity induces hyporesponsiveness and can inhibit production of humeral blocker antibodies as immunological regulators to maintain the pregnancy. Anti-paternal cytotoxic antibodies (APCAs), anti-idiopathic antibodies (Ab2), and mixed lymphocyte-reaction blocking antibodies (MLR-Bf) are a part of regulators that cover paternal HLA molecules in the surface of fetuses and make a barrier for attacking the maternal T cells and NK cells.^[1,16,18,19] Production of APCA, Ab2, and MLR-Bf

antibodies, inhibition of T lymphocytes by reducing the maternal IL-2 receptors, shifting of the Th1 to Th2 immune response, and decreasing NK cell function are beneficial effects of immunotherapy with paternal lymphocytes.^[3,4,18,20-22]

On the other hand, in an effective treatment, the white blood cells (WBC) cross-match test becomes positive, which is a promising indicative of a high chance of pregnancy. Increased pregnancy outcome was observed after immunotherapy with paternal lymphocyte in RSA patients by many research groups in the world^[20,21,23-26] as well as in our previous investigation at Sarem Infertility Center.^[23] However, the efficacy of lymphocyte immunotherapy (LIT) for RSA was doubted by some researchers, because no beneficial effect was found after LIT.^[27,28] According to previous studies, the effectiveness of LIT in different patients is variable and the cross-match test becomes positive in some cases only.^[3,23] This difference in response to immunotherapy might be associated with various factors such as the amount of trace elements. In this research study, zinc is suggested to have a critical effect on the outcome of immunotherapy.

Zinc is an essential trace element that has multifunctional activity in the body as follows:

Cell division, muscle growth, function of the hormones, blood coagulation, hair and nails growth, immune system function, menstrual disorders in women regeneration, reproduction, and fertility in men. There is a complicate relationship between zinc and the immune system.^[29-35]

Because of the considerable impact of zinc on specific and nonspecific immune responses, its deficiency can lead to reduction of appropriate responses.^[36-43]

Regarding the importance of APCA production after LIT, we tried to investigate the relationship between serum zinc level and the success of LIT in RSA patients.

MATERIALS AND METHODS

Patients

Six hundred and thirty-five age-matched patients with a history of miscarriage, who referred to Sarem women's hospital, were evaluated during a 10-month period. The exclusion criteria were abnormal karyotype of both parents, impaired glucose tolerance test, any anatomical abnormalities, intrauterine adhesions, cervical incompetence revealed during hysterosalpingography, positive toxoplasmosis serology, any thyroid dysfunction, any abnormality in luteal phase, prolactin level, and presence of anti-nuclear factor or anticardiolipin antibodies. Finally, 240 women who tested negative or normal to all the above screening tests and had a history of three or more

consecutive abortions were included in the study. Also, in this study, 70 normal women age-matched with the RSA patients without any history of abortion having 1-3 children, who had referred for checking up, were studied as healthy controls.

After LIT, we divided the patients with RSA into two groups: Group (a) included those who responded to the LIT and showed a positive cross-match test ($\geq 30\%$). Group (b) included those who had no response to LIT with a negative cross-match test result ($< 30\%$).

This study was approved by the ethics committee at Immunology, Asthma and Allergy Research Institute and Ethics committee at Sarem women's hospital. Also, all subjects completed consent form for this study.

Immunization procedure

This procedure was described by Orgad *et al.*^[21] Briefly, paternal peripheral blood mononuclear cell (PBMC) were prepared from 20 mL heparinized blood in sterile conditions.

The $80-85 \times 10^6$ PBMC were separated by Ficoll-Hypaque density centrifugation (Cederland, Canada). Then, the PBMCs were resuspended in 4 mL of 0.9% Hartman's solution. The aliquots of 0.5 mL were injected twice subcutaneously in a 3-week interval into forearm or arm. Two weeks after the second immunization, the patients' sera were collected and tested for APCA and serum zinc levels.

A cross-match test between maternal undiluted fresh serum and paternal fresh peripheral PBMCs was performed for the measurement of APCA. Cross-match result was considered positive when the antibodies in maternal serum could react and kill paternal PBMCs at a proportion of more than 30% in comparison to negative serum. Cell viability was assessed by counting the eosin-stained cells.^[23]

Determination of serum zinc

Serum samples were evaluated in terms of zinc concentration using GBC Atomic Absorption Spectrophotometer Systems (Victoria, Australia).

All samples and standards were analyzed in duplicates. The accuracy of the procedure was evaluated by analyzing commercially available samples of lyophilized human serum trace element, seronormTM, level 1 and 2 (Seronom, UK).

Statistical analysis

Kolmogorov-Smirnov test was carried out for assessing normal distribution. Differences in zinc concentration between groups were analyzed with the Mann-Whitney U-test. $P < 0.05$ was considered significant. The data were

expressed as means \pm SD. Statistical analysis was done using SPSS16 Inc.

RESULTS

In the present study, 240 females with RSA and the mean age of 31.76 ± 5.64 years were selected. The cross-match test was positive in 86 patients with a mean age of 31.6 ± 5.06 years (21-42 years) and negative for 154 patients with a mean age of 31.84 ± 5.96 (20-49 years).

The mean concentration of serum zinc levels in group (a) was 74.98 ± 11.88 $\mu\text{g}/\text{dl}$, which was significantly higher than those in group (b) with the concentration of 64.22 ± 9.22 $\mu\text{g}/\text{dl}$ ($P < 0.001$).

Also, serum zinc level in healthy controls with a mean age of 32.37 ± 5.76 years and without any history of abortion was 82.90 ± 12.36 $\mu\text{g}/\text{dl}$.

In Figure 1, we compared serum concentrations of zinc in group (a) (APCA positive) and group (b) (APCA negative) to those of normal subjects (normal). Our results showed that serum zinc level in group (b) was significantly lower than that in group (a) and healthy controls. As we indicated in Figure 2, there was a significant and positive correlation ($r = 0.462$) between serum zinc level and APCA production in patients treated with paternal PBMC. Patient with lower levels of zinc could not produce APCA as an indicative of humoral immune response to immunotherapy.

DISCUSSION

Several different studies have investigated the effect of zinc supplementation on the function of the immune system, especially production of specific antibodies after vaccination. Kreft *et al.*, and Provinciali *et al.*, showed that immune response to vaccination dependent on the zinc status in patients. In those patients, non responders had lower serum zinc levels and supplementation with zinc could elevate the amount of antibody after vaccination.^[37,44,45] Izdebska-Szymona *et al.*, reported the effect of zinc on humoral and cellular response in mice and found that anti-SRBC antibody levels was greater in supplemented mice than in control ones.^[40] Zinc deficiency can affect the immune response and the production of antibodies. Because the aim of LIT is production of blocker antibodies such as APCA, zinc deficiency can affect it.

Chaichian *et al.*, in Sarem Infertility Center followed-up 93 patients with positive cross-match test after LIT. In this study, pregnancy occurred in 49 of 93 patients, which was clinically successful in about 52.7% of them.^[23] In the present study, among 86 patients with positive cross-match test, we could follow-up 74 patients. Of them, 51 (68.9%) patients were revealed to have documented pregnancy and, among them, successful clinical pregnancy (gestational age of at least 28 weeks) were recorded in only 38 subjects. Therefore, successful clinical pregnancy was reported in 51.3% (38/74) of those with positive APCA. In present study, was observed that different patients have variable responses to this kind of immunotherapy and the cross-match test becomes negative

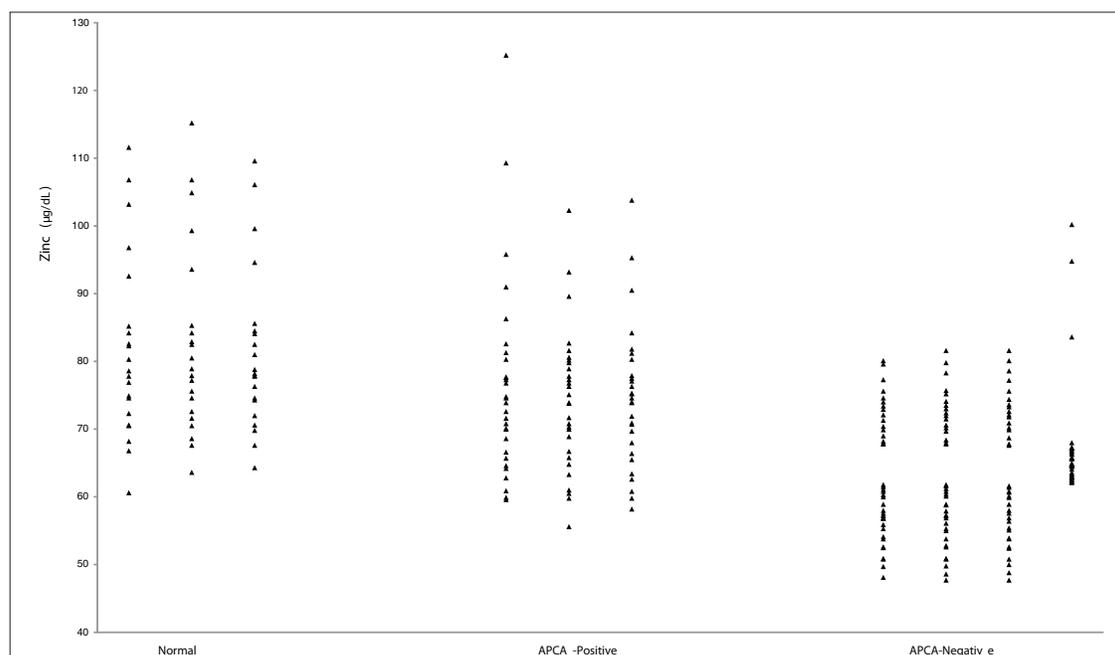


Figure 1: Serum zinc levels in normal subjects (normal) and two groups of RSA patients: Group (a) (APCA positive) responded to the paternal lymphocytes and their cross-match test was positive and group (b) (APCA negative) did not respond to paternal lymphocytes immunizations and their cross-match test was negative

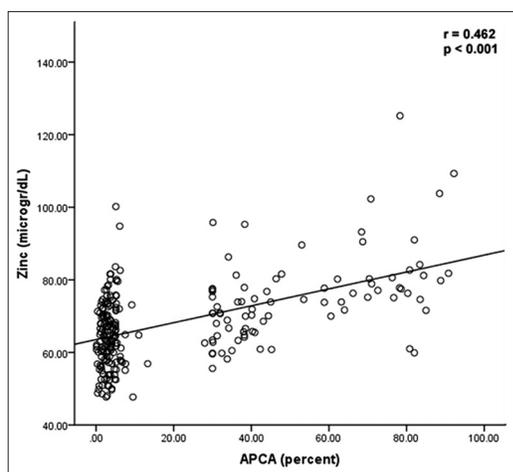


Figure 2: Correlation between serum zinc level and APCA production in patients treated with paternal PBMC; patient with zinc deficiency could not respond to immunotherapy

in most cases (154 patients). On the other hand, some studies reported that LIT for unexplained RSA had no beneficial effect,^[27,28] and, until date, the use of LIT for the treatment of RSA remains a matter of controversy. Lack of response to LIT may be associated with the amount of zinc.

To the best of our knowledge, there has been no study on correlation between serum zinc level and success of LIT in RSA. Apgar *et al.*, showed that zinc intake affects the maintenance of guinea pig pregnancy.^[46] Also, Graham *et al.*, in 2009 demonstrated that serum zinc and copper concentrations had a positive correlation with spontaneous abortion in cows.^[47] In this study, patients with RSA who responded to LIT due to their positive cross-match had higher serum zinc levels than those with negative cross-match test. In other words, LIT could not be effective for induction of APCA production in zinc-deficient patients.

In this study, we also revealed that serum zinc levels were higher in normal age-matched group of women than both groups of patients (with positive and negative cross-match results).

In conclusion, supplementation of RSA patient with zinc before LIT is highly recommended and would be a promising strategy to produce more blocking antibodies after immunotherapy toward keeping the fetus in patients with RSA.

ACKNOWLEDGMENTS

This work was supported by research grants from Tehran University of Medical Sciences and Health Services, Immunology, Asthma and Allergy Research Institute and the Sarem Women's Hospital, Sarem Cell Research Center, Tehran, Iran. The authors report no conflicts of interest.

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How to cite this article: Zare A, Saremi A, Hajhashemi M, Kardar GA, Moazzeni SM, Pourpak Z, Salehian P, Naderi M, Safaralizadeh R, Nourizadeh M. Correlation between serum zinc levels and successful immunotherapy in recurrent spontaneous abortion patients. *J Hum Reprod Sci* 2013;6:147-51.

Source of Support: Tehran University of Medical Sciences and Health Services, Immunology, Asthma and Allergy Research Institute and the Sarem Women's Hospital, Sarem Cell Research Center, Tehran, Iran,

Conflict of Interest: None declared.